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Immature stages and biology of Bornean *Arhopala* butterflies (Lepidoptera, Lycaenidae) feeding on myrmecophytic *Macaranga*

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Abstract We examined the immature stages, larval behaviors, host plant selection, and parasitoids of four Bornean *Arhopala* butterflies feeding on *Macaranga* plants which often have species-specific relationships with symbiotic ants. We then compared their biological characteristics to those of butterflies on the Malay Peninsula reported in a previous study. At our study site, the larvae of three *Arhopala* species were found on *Macaranga myrmecophytes*. Each *Arhopala* fed on one or two closely-related *Macaranga* species: *A. amphimuta* fed on *M. trachyphylla* and *M. bancana*, *A. zylda* fed on *M. beccariana* and *M. hypoleuca*, and *A. dajagaka* fed on *M. hosei*. The butterfly-plant relationships and the species-specificity were similar to those observed on the Malay Peninsula. The other Arhopala species, *A. major*, was observed feeding on non-myrmecophytic *M. gigantea* and *Macaranga* sp. A. Among the four Macaranga-feeding *Arhopala* species, we noted remarkable interspecific variation in larval morphology, behavior, and parasitoid composition. These variations were presumed to be associated with differences in the aggressiveness of ants on host *Macaranga* plants.

Key words *Arhopala*, ant-association, ant-plant, evolution, Lycaenidae, *Macaranga*, mutualism, myrmecophily, myrmecophytes, parasitoids.

Introduction

Arhopala Boisduval, a lycaenid genus, includes over 200 species that are distributed in sub-tropical to tropical zones of the Indo-Australian region (Megens *et al.*, 2004). Adult *Arhopala* are generally found in primary lowland forests, but some species occur in secondary vegetation, coastal mangroves, and woody savannahs (Common & Waterhouse, 1981; Parsons, 1998). The larvae of most *Arhopala* species are associated with ants and feed mainly on Fagaceae and Euphorbiaceae (Fiedler, 1991; Robinson *et al.*, 2001; Megens *et al.*, 2005). Of these, the *A. amphimuta* subgroup (sensu Evans 1957 and Eliot 1963, 1992), which is composed of approximately ten species, uses *Macaranga* species (Euphorbiaceae) as its host plants (Maschwitz *et al.*, 1984; Megens *et al.*, 2005).

The genus *Macaranga* includes more than 20 species of myrmecophytes (Whitmore 1969; Quek *et al.*, 2004). These myrmecophytic species often develop mutualistic relationships with species-specific specialist ants of the genera *Crematogaste* or *Camponotus* (Fiala & Maschwitz, 1991, 1992; Fiala et al., 1989, 1999; Maschwitz *et al.*, 1996; Federle *et al.*, 1998). These plants provide the symbiotic ants with nesting space in the internodes, and with food bodies on the surface of leaves or stipules (Fiala *et al.*, 1989; Fiala & Maschwitz, 1991). When the plants are damaged by herbivory, the ants attack and remove the herbivores.

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Although myrmecophytic *Macaranga* species are well defended by their symbiotic ants (Fiala *et al.*, 1994, Itioka *et al.*, 2000), a few *Arhopala* species are known to feed on such plants, probably by evading the aggressive ants through chemical and behavioral mechanisms (Maschwitz *et al.*, 1984). To study how the larvae of *Arhopala* species have adapted to the anti-herbivore defenses by symbiotic ants of *Macaranga myrmecophytes* is very interesting and is important for understanding the evolution of myrmecophily or ant-association in Lycaenidae. However, few studies have given details of behavior in myrmecophyte-feeding *Arhopala* species which moght have evolved to tame symbiotic ants, or of feeding behaviors and host plant preferences (Maschwitz *et al.*, 1984). Moreover, the immature stages of the butterflies have not been well studied.

The geographic distributions of *Arhopala* and *Macaranga* are well known (Evans, 1957; Whitmore, 1969; Fiala et al., 1989; Parsons, 1998), but to date, studies of the host plant utilization of *Macaranga* by *Arhopala* have been restricted to the Malay Peninsula. Only three *Macaranga* species have been investigated, and each of these species serves as a host for the larvae of one *Arhopala* species (Maschwitz *et al.*, 1984). In addition, the geographical variation of *Macaranga* utilization by *Arhopala* has never been examined.

Here we describe the morphological and biological characteristics of the immature stages of *Arhopala* species using mainly myrmecophytic *Macaranga* species in Borneo, where the highest species richness of *Macaranga* is found (Whitmore, 1981). We also compared *Macaranga* utilization by *Arhopala* between Borneo and the Malay Peninsula and discuss the geographical variation of the species.

Materials and methods

Materials

We studied four *Arhopala* butterflies feeding on *Macaranga* plants. The scientific names of these species and their symbiotic ants are provided in the Results section. The species of parasitoids were identified after they emerged from the larvae or pupae.

The body lengths of first- or final-instar larvae of the *Arhopala* species were measured just before diapause. The body segments and myrmecophilous organs in *Arhopala* species are abbreviated as follows: abdominal segment=AS, dorsal nectary organ=DNO, tentacle organs=TOs, pore cupola organs=PCOs.

At least 16 *Macaranga* species, including about 11 myrmecophytic species, were available as possible host plants for *Arhopala* species in our study site (Itioka, 2005). Of these, we targeted 11 myrmecophytic and five non-myrmecophytic *Macaranga* species (Table 1). Of the myrmecophytes, *M. trachyphylla* and *M. bancana* are closely related to one another, as were *M. beccariana* and *M. hypoleuca* (Davies *et al.*, 2001).

Study site

Our study was conducted in Lambir Hills National Park, Sarawak, Malaysia (4°2′N, 113°2′E, 150 to 200 m a.s.l.). Consistently high temperatures with no distinct dry season characterize the climate of the region. The mean annual temperature and annual precipitation are 26°C and 2,700 mm (Nakagawa *et al.*, 2000). This park is covered primarily with lowland mixed dipterocarp forest.

Sampling and rearing

To determine which species of *Arhopala* feeds on which species of *Macaranga*, we searched

Table 1. Censused *Macaranga* species and the numbers of trees surveyed for the presence of immature *Arhopala* species during the three survey periods.

Macaranga species	myrmecophytes/ non-myrmecophytes	Number of surveyed trees			
		Total	August 2006	January 2007	August 2007
M. winkleri	myrmecophytes	105	36	27	42
M. trachyphylla	myrmecophytes	225	78	78	69
M. bancana	myrmecophytes	224	77	65	82
M. beccariana	myrmecophytes	248	73	93	82
M. hosei	myrmecophytes	39	11	13	15
M. hypoleuca	myrmecophytes	51	13	14	24
M. havilandii	myrmecophytes	20	4	4	12
M. hullettii	myrmecophytes	76	28	22	26
M. lamellata	myrmecophytes	30	8	10	12
M. kingii	myrmecophytes	29	7	10	12
M.. psedopruinosa	myrmecophytes	66	30	22	14
M. gigantea	non-myrmecophytes	76	35	24	17
M. conifera	non-myrmecophytes	20	9	6	5
M. reculvata	non-myrmecophytes	7	3	3	1
M. praestans	non-myrmecophytes	65	21	23	21
Macaranga sp. A	non-myrmecophytes	6	2	2	2

for the eggs, larvae, and pupae of *Arhopala* species on *Macaranga* plants in the field. Sampling was conducted in August 2006, January 2007, and August 2007 (Table 1). When *Arhopala* species were found, we recorded their locations and feeding traces on the host plants, ant behavior toward the larvae, and the number of attendant ants. Next we collected eggs, larvae, and pupae and reared them individually in plastic boxes (180×120×100 mm) at the Lambir Japan Laboratory. Until they reached the pupal stage, larvae were fed the apical part of their host plants, which consisted of a piece of stem with a few young leaves. The food sections were randomly selected to avoid herbivory damage in the field, and were replaced every 2 days. The end of the stem was inserted into a sponge used for floral arrangements (KYUSUI SUPONGI: DAISO CO. LTD.). The growth of the eggs, larvae, and pupae was monitored every day until they reached the adult stage.

Results

In total, we found 27 eggs and 108 larvae of four *Arhopala* species from five myrmecophytes (*Macaranga trachyphylla*, *M. bancana*, *M. beccariana*, *M. hosei*, and *M. hypoleuca*) and two non-myrmecophytes (*M. gigantea* and *Macaranga* sp. A.; Fig. 1). Each of the seven *Macaranga* species hosted larvae of a single *Arhopala* species as follows: *M. trachyphylla* and *M. bancana* were host plants of *Arhopala* amphimuta, *M. beccariana* and *M. hypoleuca* were host plants of *A. zylda*, *M. hosei* was a host of *A. dajagaka*, and *M. gigantea* and *Macaranga* sp. A were hosts of *A. major*. The detailed descriptions of the immature stages of the four *Arhopala* species are as follows:

Arhopala amphimuta (C. & R. Felder, 1860) (Figs 2–10)

Eggs. Eggs were approximately 1 mm (n=3) in diameter, white, hemispherical, and covered with rugged echinulate processes (Fig. 2). They were laid singly on the abaxial side of young leaves near the apical part of the stem.

Larvae. Larvae were onisciform as in most lycaenids, somewhat flattened, and exhibited body color variation in each instar. First-instar larvae were approximately 2 mm (n=1) in

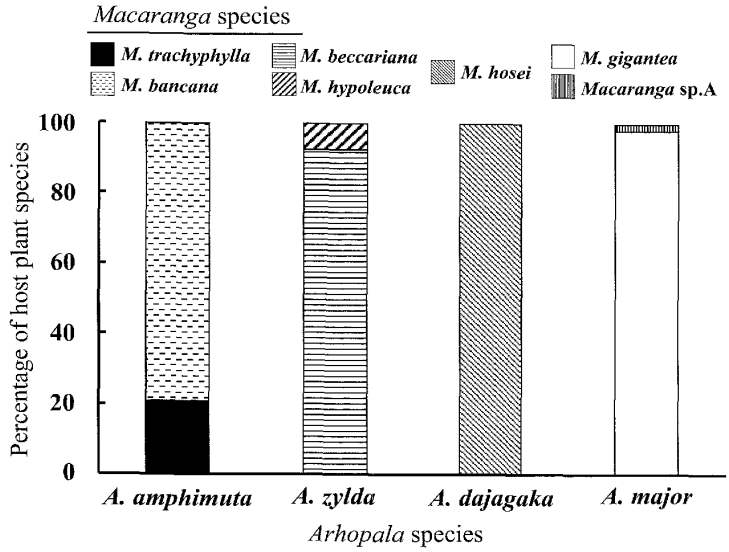
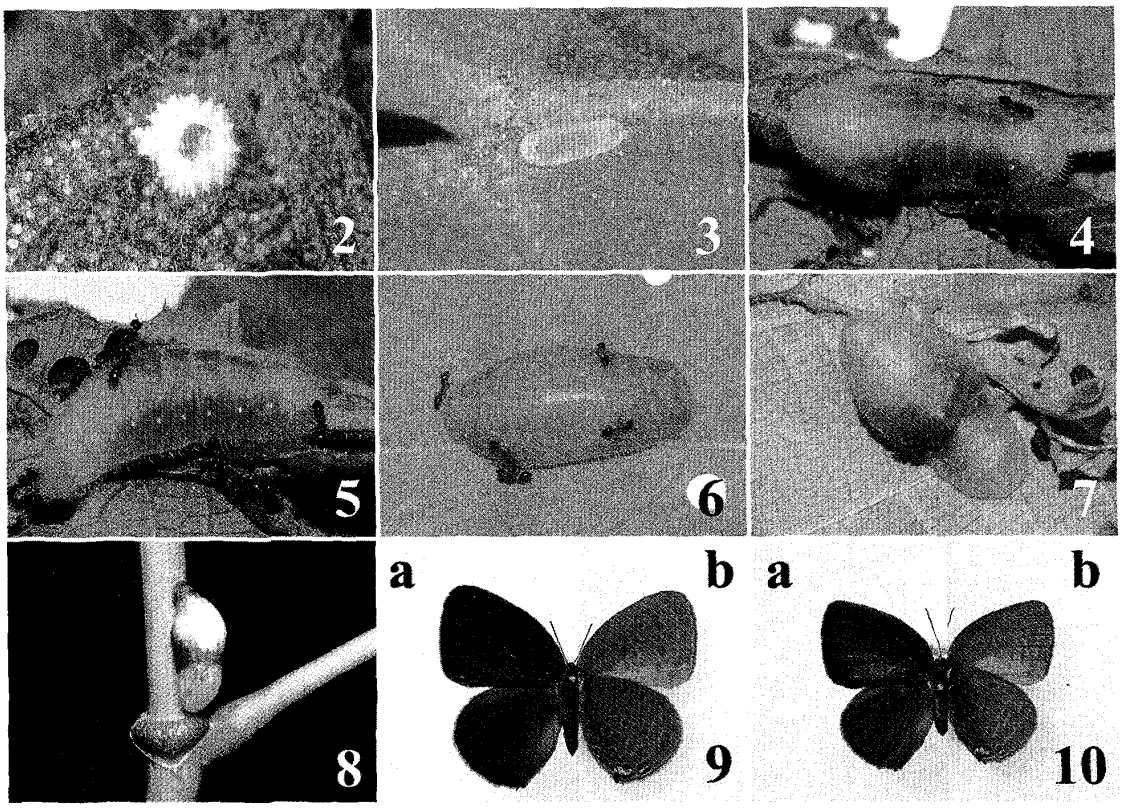


Fig. 1. Host preference of four *Arhopala* species feeding on *Macaranga* plants in Lambir, Borneo.

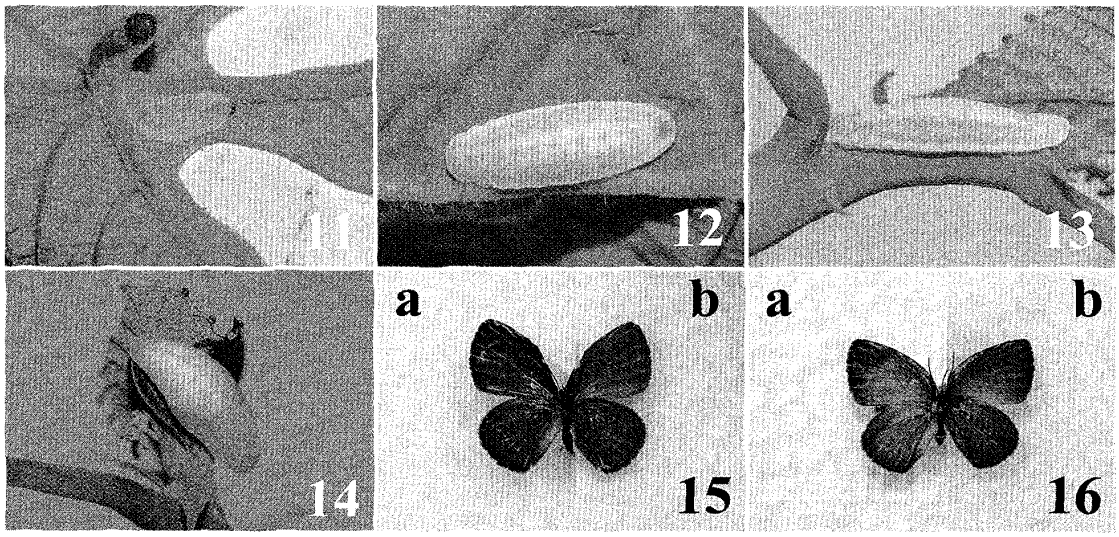
body length and pale greenish (Fig. 3). The second to final (fourth) instars showed a series of body color variations between two extreme types, *i. e.*, from evenly light green (green type) to light green with brownish red on the dorsal portion of AS2–6 and on the dorsolateral portion of AS1–7 (red type). Larvae of the green type tended to rest on light green leaves (Fig. 6), and red type larvae were mostly found on brownish red leaves (Figs 4, 5). When reared in the laboratory, the larvae changed into the green or red type according to the color of the fresh leaves they fed on (Figs 3–7). In the field, they rested mainly on the abaxial side of young leaves (the inner side of curled leaves) throughout the larval stage, although the final instars were often found on the apical parts of the stem near stipules, and were always seen raising the anterior half of their AS, as if to mimic the stipules (Fig. 7). They made holes and large feeding marks when feeding on young leaves, and the first instars formed an irregular patchwork of feeding marks and did not disturb the upper or under epidermis of the leaf. From hatching to pupation, one larva usually consumed just less than one full young leaf. From the second to final instars, the larvae exhibited a DNO on the middorsal portion of AS7, a pair of TOs on the dorsolateral portion of AS8, and PCOs throughout the body surface. The final-instar larvae in particular developed these myrmecophilous organs, which were attended to by symbiotic ants of the host plants. The final instars were 16–17 mm (n=4) in body length and were often observed secreting honeydew from the DNO. The droplets of honeydew from final-instar larvae never exceeded 0.5 mm in diameter, and the average appeared to be much smaller.

Pupae. The pupae measured 12–13 mm (n=2) in length, and were smooth, squat, and gourd-like dorsally but flattened ventrally. The end of the abdomen was somewhat swollen and rounded, similar to other Arhopala species. Irrespective of body color in the larval stages, the pupae were light green on the head, the thorax, and the wing sheaths of the anterior third; brownish red on AS1–2, the wing sheaths of the posterior middle third, and the ventral portion and the end of the AS; and yellowish green on the remaining third of the AS (Fig. 8). Pupation took place on a leaf stalk (base of a leaf blade) around the apical part of the host plant, irrespective of the height of host plants. The symbiotic ants of the host plants often attended the pupae.

Host plants. All larvae in our study site were found on *M. trachyphylla* and its allied *M. bancana* (both myrmecophytes).



Figs 2–10. Immature and adult *Arhopala amphimuta*. 2. Egg after hatching. 3. First-instar larva on the abaxial surface of a young leaf, dorsal view. 4. Final-instar larva (red type), dorsal view. 5. Final-instar larva (red type), lateral view. 6. Final-instar larva (green type), dorsal view. 7. Final-instar larva (red type) raising the anterior half of its abdominal segments. 8. Pupa on a stem of a host plant, lateral view. 9. Male adult, top (a) and underside (b). 10. Female adult, top (a) and underside (b).



Figs 11–16. Immature and adult *Arhopala zylda*. 11. First-instar larva on the abaxial surface of young leaf, dorsal view. 12. Final-instar larva, dorsal view. 13. Final-instar larva, lateral view. 14. Pupa on an apical leaf of host plant, dorsolateral view. 15. Male adult, top (a) and underside (b). 16. Female adult, top (a) and underside (b).

Ants. The closely related ant species of the *Crematogaster borneensis* group (*C. borneensis* L1 and L2 in Itino *et al.*, 2001 and probably Msp.2 and Msp.3 in Fiala *et al.*, 1999), symbiotic ants of *M. trachyphylla* and *M. bancana*, were observed attending larvae. An average of 6.8±0.97 (mean±SE, N=5, data not shown) ants attended one final-instar larva.

Parasitoids. Of the collected larvae, 34% were parasitized by *Aplomya distincta* (Tachinidae, Diptera) or *Xanthopimpla pumilio* (Ichneumonidae, Hymenoptera). In both parasitoid species, only one larva emerged from one host. However, the two parasitoids emerged at different growth stages of the hosts. Specifically, *A. distincta* emerged from the host prepupa, whereas *X. pumilio* emerged from the host pupa.

Arhopala zylda Corbet, 1941 (Figs 11–16)

Eggs. Unknown.

Larvae. Larvae of *A. zylda* differed from those of *A. amphimuta* as follows. First-instar larvae were 1.8 mm (n=1) in length and evenly yellowish-white. Overall, the second to final instars were light yellow, but the middorsal portion was paler throughout the length of the larva (Figs 11–13). The larvae rested on the abaxial side of young leaves and fed on young leaves as well as food bodies secreted from the abaxial side of a few apical young leaves. The final instars were 12–13 mm (n=4) in length and developed paired TOs but no DNO.

Pupae. The pupae of *A. zylda* were similar to those of *A. amphimuta*, but differed as follows. Pupae were 9–10 mm (n=2) in length and more slender as a whole. The body color was light green on the head, thorax, and wing sheaths, and the abdomen was whitish-yellow (Fig. 14). The pupation site was on the apical part of the stem of the host plants, irrespective of the height of the host plants.

Host plants. All *A. zylda* larvae collected at our study site were found on *M. beccariana* and *M. hypoleuca* (both myrmecophytes).

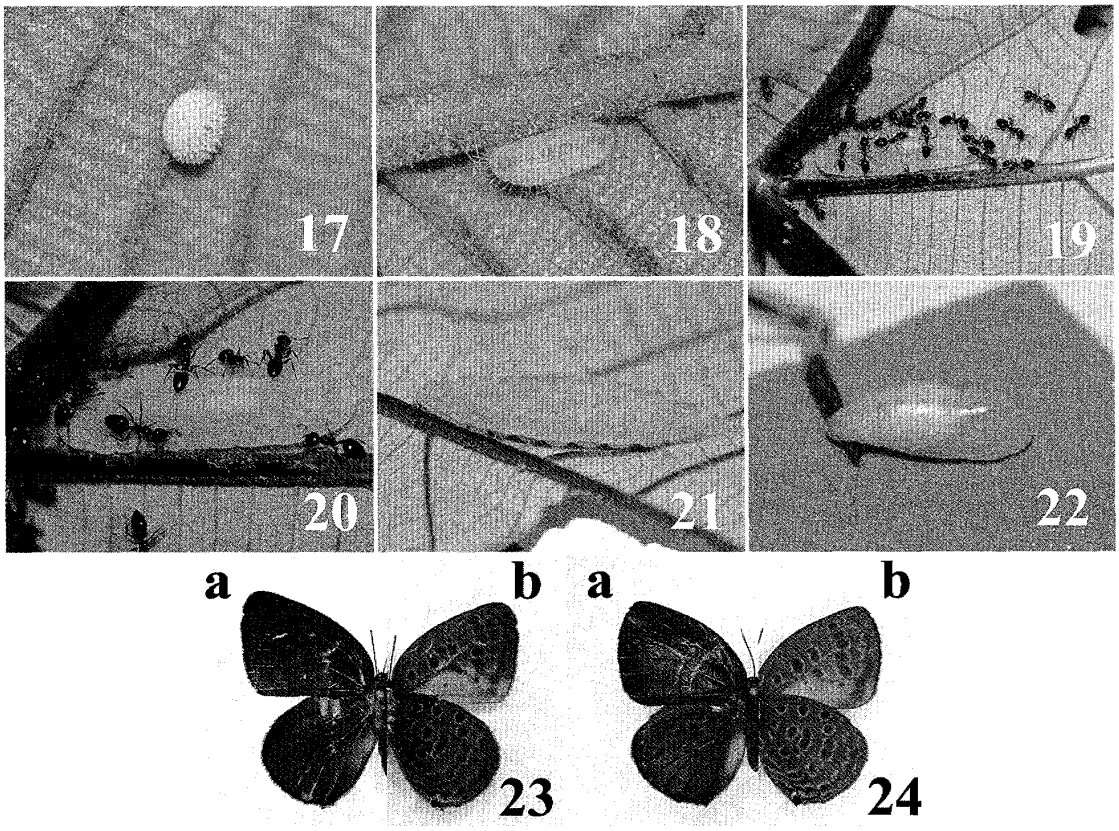
Ants. Workers of *Crematogaster decamera* Forel (Itino *et al.*, 2001; probably Msp.5 in Fiala *et al.*, 1999), a symbiotic ant species of *M. beccariana* and *M. hypoleuca*, attended the *A. zylda* larvae, but the frequency of attendance was very low, and attending ants were rarely observed.

Parasitoids. Of the collected larvae, 29% were parasitized by either *Aplomya distincta* or *Xanthopimpla pumilio*. The number of emerging parasitoids per host, the growth stages of parasitoids at the time of parasitoid emergence, and the growth stage of the host at that time were all the same as observed for the parasitoids of *A. amphimuta* (see above).

Arhopala dajagaka Bethune-Baker, 1896 (Figs 17–24)

Eggs. Eggs of *A. dajagaka* were approximately 1 mm (n=3) in diameter and very similar to the eggs of *A. amphimuta* in color and shape, but *A. dajagaka* differed in having somewhat smaller echinulate processes (Fig. 17). As in the case of *A. amphimuta*, the adult *A. dajagaka* females deposited eggs singly on the abaxial side of young leaves.

Larvae. The larvae of *A. dajagaka* were quite similar to those of *A. zylda*, but differed in the following characteristics. First-instar larvae were whitish yellow and 2.3 mm (n=1) in length (Fig. 18). From the second to final instars, the larvae were pale green (Figs 19–21). The larvae rested on the underside of a young leaf to feed, chewing its margin or making holes in its inner area. From hatching to pupation, one larva fed on two or three young leaves from the apical part of a stem. The final-instar larvae were 24–25 mm (n=4) in body



Figs 17–24. Immature and adult *Arhopala dajagaka*. 17. Egg on the abaxial surface of a young leaf. 18. First-instar larva on the abaxial surface of young leaf, dorsal view. 19. Final-instar larva attended by several symbiotic ants of *Macaranga hosei*. 20. Final-instar larva, dorsal view. 21. Final-instar larva, lateral view. 22. Pupa on sponge used for the laboratory rearing, dorsolateral view. 23. Male adult, top (a) and underside (b). 24. Female adult, top (a) and underside (b).

length and developed a DNO and paired TOs. They were often observed secreting honeydew droplets of >1 mm in diameter from the DNO.

Pupae. The pupae of *A. dajagaka* were very similar to those of *A. zylda* in color and shape, but differed as follows. Pupae were 15–18 mm (n=2) in length, slightly darker, and somewhat broader in the head and thorax (Fig. 22). The pupation site was on the leafstalk of a leaf near the apical part of the host plant, irrespective of the height of the host plants. During pupation, the pupa secluded itself from the outside by weaving a leaf margin around the base of the leaf blade. The symbiotic ants of the host plants often attended the pupae.

Host plants. All individuals in the immature stages of *A. dajagaka* were found on the myrmecophyte *M. hosei* in the field.

Ants. Workers of the symbiotic ant species of *M. hosei*, *Crematogaster* sp. 4 (Itino *et al.*, 2001 and probably Msp.1 in Fiala *et al.*, 1999) attended the larvae of *A. dajagaka*. An average of 17.5±2.72 (mean±SE, N=4) ants attended a final-instar larva (data not shown).

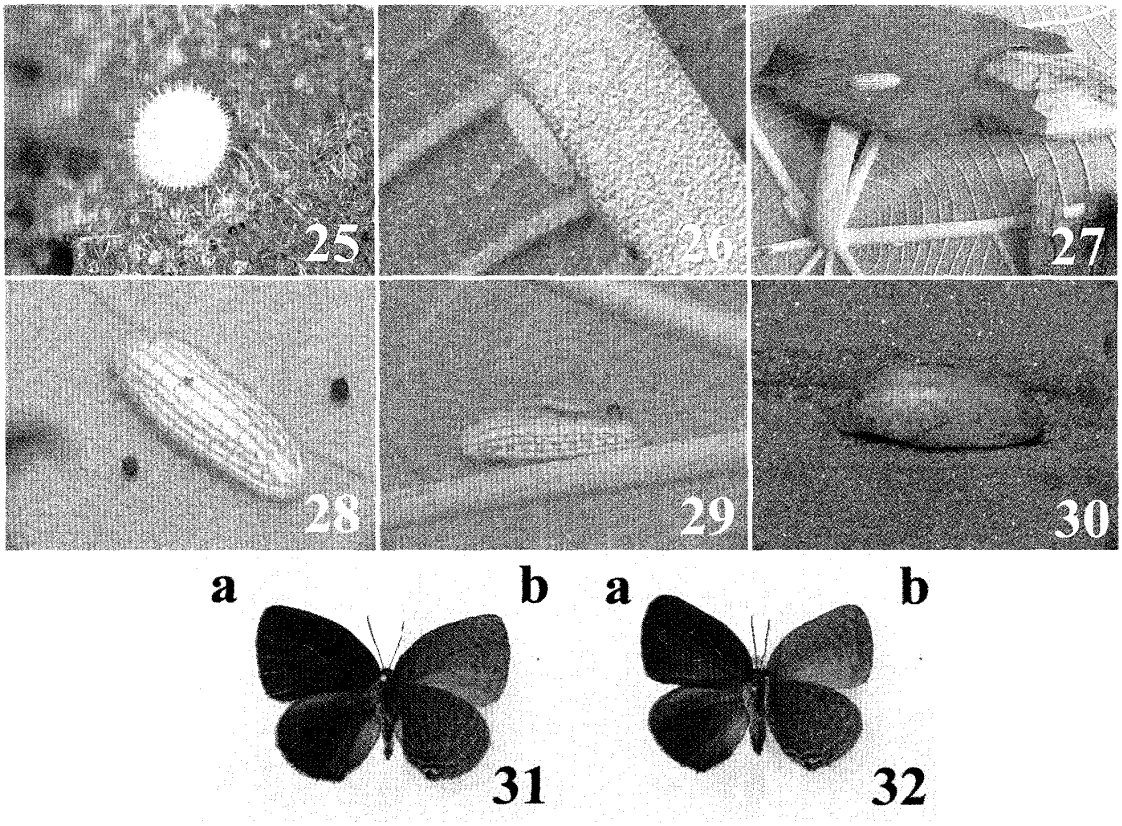
Parasitoids. Of the collected larvae, 33% were parasitized by *Aplomya distincta* or *Xanthopimpla pumilio*. The two parasitoids showed similar life-history characteristics to those that parasitized *A. amphimuta* or *A. zylda* (see above).

Arhopala major Staudinger, 1889 (Figs 25–32)

Eggs. Eggs of *A. major* were approximately 1 mm (n=3) in diameter and resembled those of *A. amphimuta* and × in color and shape, but differed in that they were more slender and exhibited abundant echinulate processes (Fig. 25). As in *A. amphimuta* and *A. dajagaka*, the adults deposited their eggs singly on the abaxial side of young leaves.

Larvae. The larvae of *A. major* differed from those of *A. zylda* and *A. dajagaka* in the following characteristics. First-instar larvae measured 2.2 mm (n=1) in length and were evenly pale green. Most of the newly hatched larvae rested on the undersides of young leaves. Following the first instar, the body color was pale yellow with one middorsal, two dorsolateral, and one spiracular dull green line (Figs 26–29). The larvae usually rested on the adaxial side of a stipule, in a cavity between the stem and the stipule, and concealed themselves from the outside (Fig. 27). We consistently found several feeding marks that were probably made by the larvae on young leaves near the stipule that housed the final instar. Final-instar larvae were 19–21 mm (n=4) in length. The older larvae developed a DNO and paired TOs. Honeydew droplets from the DNO never exceeded 0.5 mm in diameter.

Pupae. Pupae were very similar to those of *A. amphimuta* in shape, but differed in size and color as follows. The body length was 14–15 mm (n=2), and the overall body color was



Figs 25–32. Immature and adult *Arhopala major*. 25. Egg on the abaxial surface of young leaf. 26. First instar larva on the abaxial surface of young leaf, dorsal view. 27. Second-instar larva on the abaxial surface of young leaf, and third-instar larvae on the adaxial side (inside) of stipule. 28. Final-instar larva, dorsal view. 29. Final-instar larva, lateral view. 30. Pupa on sponge used for the laboratory rearing, dorsolateral view. 31. Male adult, top (a) and underside (b). 32. Female adult, top (a) and underside (b).

ocherous with fine stains and a whitish abdomen (Fig. 30). The pupation site was found on the apical part of a stem covered with stipules, irrespective of the height of the host plants.

Host plants. One larva was from *Macaranga*. sp. A, and all others were from *M. gigantea* (both non-myrmecophytes).

Ants. At least two species of non symbiotic ants of *Macaranga*, including *Crematogaster* sp. and *Dolichoderus* sp., attended these larvae, but the frequency and intensity of attendance was lower than those for the other *Arhopala* species found on myrmecophytic Macaranga species. The larvae resting on stems covered with stipules were not attended by ants, whereas those feeding on leaves were attended by one or two worker ants on several occasions. In such cases, the workers often left the larvae alone on the leaves.

Parasitoids. Of the collected larvae, 39% were parasitized by *Apanteles* sp. (Braconidae, Hymenoptera) or *Aplomya distincta*. The latter parasitoid showed similar life-history characteristics to those parasitizing *A. amphimuta*, *A. zylda*, and *A. dajagaka* (see above). The former parasitoid differed from the latter in that it emerged during the middle-instar larval stage of the host, although the number of parasitoids per host and the growth stage of the parasitoid at the timing of emergence was the same as the former.

Discussion

Species-specificity and relationships between *Arhopala* and *Macaranga*

Our data demonstrated that, except for the non-myrmecophyte-feeding by *A. major*, each of three *Arhopala* species fed on one or two closely related *Macaranga myrmecophytes*. Specifically, *A. amphimuta* fed on *M. trachyphylla* and *M. bancana*, *A. zylda* fed on *M. beccariana* and *M. hypoleuca*, and *A. dajagaka* fed on *M. hosei*. These observations are almost consistent with the findings of a study conducted in the Malay Peninsula by Maschwitz *et al.* (1984), who reported that a single *Arhopala* species fed on a single species of *Macaranga myrmecophyte*, *i. e.*, *A. amphimuta* fed on *M. triloba*, *A. zylda* on *M. hypoleuca*, and *A. moolaiana* (Moore, 1879) on *M. hullettii*.

Comparing the relationships between the host plants of myrmecophyte-feeding *Arhopala* butterflies in these two areas, the two host plants of *A. amphimuta* from Borneo, *M. trachyphylla*, and *M. bancana*, are not very closely related to *M. triloba* from the Malay Peninsula, which is widely distributed in Malaysia and its surroundings but not in Borneo (Davies, 2001). Nevertheless, host specificity is quite high in smooth-stemmed species (including all host plants of *A. amphimuta*), which is a monophyletic group characterized by non-waxy, smooth stems that belongs to the *Macaranga pachystemon* group (Quek *et al.*, 2004). Moreover, the plant-ants living with the three *Macaranga* species are identical and include only two species of the *Crematogaster borneensis* group (Itino *et al.*, 2001; Fiala *et al.*, 1999). *Arhopala zylda* feeds on *M. hypoleuca* in the Malay Peninsula and Borneo, and also feeds on *M. beccariana*, which is sister to *M. hypoleuca* and is endemic to Borneo (Davies *et al.*, 2001). In addition, the symbiotic ant *Crematogaster decamera* is common to the two host plants.

Although *A. moolaiana*, which feeds on *M. hullettii* in the Malay Peninsula, was not confirmed at our study site, some circumstantial evidence suggests that *A. moolaiana* might also uses *M. hullettii* as a host in Borneo. First, adults of *A. moolaiana* were often found at our site (Itioka *et al.*, unpublished data), and second, several individuals of lycaenid larvae that seemed to belong to *Arhopala* have been found feeding on *M. hullettii* at our site (Itioka, unpublished data). However, whether *A. moolaiana* indeed feeds on *M. hullettii* re-

mains to be investigated.

Interestingly, we observed that the host plant of the Borneo-endemic *Arhopala dajagaka* (Seki *et al.*, 1991) is the myrmecophyte *M. hosei* and that the symbiotic ants are from only one species, *Crematogaster* sp. 4 (Itino *et al.*, 2001). Despite our intensive survey on more than 1,200 individuals of 16 *Macaranga* species (Table 1), the larvae of *A. dajagaka* were only discovered on *M. hosei*. This suggests that *A. dajagaka* may be a specialist on *M. hosei*.

Our data suggest that species-specificity in herbivore-host plant relationships between myrmecophyte-feeding *Arhopala* butterflies and their host *Macaranga* is as high in Borneo as it is in the Malay Peninsula. Moreover, the relationship among *Arhopala* butterflies, myrmecophytic *Macaranga* plants, and symbiotic ants is also likely to be similar in the two areas. Conversely, the larvae of *A. major* in our study site fed on the non-myrmecophitic *M. gigantea* and *Macaranga* sp. A, which differed notably in their morphology and life history from each other. In addition, these species appear to be distantly related to each other (data not shown). Megens *et al.* (2005) also noted that *A. major* feeds on non-myrmecophytic *Macaranga* species. The host range of this *Arhopala* species is probably wide among non-myrmecophytic *Macaranga*, and the attending ants are also variable. This indicates that *A. major* exhibits a considerably lower species-specificity and a weaker relationship with its host *Macaranga* spp. and symbiotic ants compared to the myrmecophyte-feeding *Arhopala* butterflies.

Body shape, coloration, and behavior of larvae and pupae of *Arhopala* species

All *Arhopala* species that we studied appeared to camouflage themselves in terms of larval body shape and color patterns (Figs 3–8, 11–14, 18–22, 26–30). This hypothesis does not conflict with the results reported by Maschwitz *et al.* (1984). Specifically, the color variation corresponded to that of the leaves of their host plants. For example, the young leaves of *M. trachyphylla* and *M. bancana* showed variation from green to reddish-purple, and *A. amphimuta* larvae likely benefit by making their body color similar to the color of the leaves that they rest on. Their pupae also showed the same color as the final-instar larvae. Although only the red type larvae of this species have been identified previously (Maschwitz *et al.*, 1984), the larvae and pupae of *A. amphimuta* exhibited a spectrum of color variation that corresponds to the colors of the fresh leaves on which they feed (Figs 3–7). Similarly, the larvae and pupae of *A. zylda*, *A. dajagaka*, and *A. major* appeared to exhibit specific colors similar to those of the plant tissue that they inhabited (*A. zylda*, Figs 11–14; *A. dajagaka*, Figs 18–22; *A. major*, Figs 26–30). Such ability to adapt to the color variation of the background habitat is presumed to be effective for escaping from visual sensory predators such as birds and lizards.

Our observations suggest that larvae of the three myrmecophyte-feeding *Arhopala* species select the abaxial side of young leaves as resting sites, whereas those of non-myrmecophyte-feeding *A. major* stayed in the confined space on the stem that is compartmentalized by stipules around the apical parts. The difference between the resting areas may reflect the predictability and intensity of attacks by symbiotic ants against aliens approaching the host plants. The ants that inhabit myrmecophytes are much more aggressive than those found on non-myrmecophytes. The presence of ants would be effective for helping butterfly larvae evade predators or parasitoids if the larvae could tame the ants, because ants tend to exclude enemies from the host plants (Fiedler & Maschwitz, 1989; Fiedler, 1991). Under conditions without such aggressive symbiotic ants, *A. major* may select the closed space so that it can hide from enemies.

Myrmecophilous organs of *Arhopala* species

The majority of lycaenids have coevolved with ants, and there is wide variation in associations that can be either facultative or obligate and that range from mutualism to parasitism (Pierce *et al.*, 2002). The ant associations have exerted a strong selection pressure on lycaenid larval morphology (Malicky, 1970). As a result, many lycaenid larvae develop myrmecophilous organs to protect themselves from ant attacks and/or to tame attending ants (DeVries, 1990). In particular, the honeydew-secreting organ, DNO, plays a critical role in maintenance of ant-lycaenid mutualisms (Fiedler & Maschwitz, 1988, 1989; Leimar *et al.*, 1993; Axen *et al.*, 1996).

We have described biological and morphological characteristics, including myrmecophilous organs, that were not reported in detail by Maschwitz *et al.* (1984). Older larvae of *A. amphimuta*, *A. dajagaka*, and *A. major* are usually attended by ants and possess three fundamental myrmecophilous organs, DNO, PCOs, and TOs, found in many lycaenids, while those of *A. zylda* lack the DNO but retain the other myrmecophilous organs. The PCOs exist universally in all lycaenids and secrete chemical compounds (mainly amino acids and hydrocarbons) as a defense against ants (Pierce, 1983; Fielder, 1991; Akino *et al.*, 1999). Although their precise function is unknown, the eversible TOs have been thought to produce volatile secretions (*e. g.*, mimics of ant alarm pheromones) or to visually attract the attention of ants to prevent them from attacking the lycaenid larvae (Fiedler, 1991). During our observations, no *A. zylda* larvae were attacked by aggressive ants living in symbiosis with their host plants, although they were occasionally attended by the plant-ants. This indicates that the DNO effectively provoked the ants to attend the larvae, but that TOs and PCOs are more important than the DNO for controlling the aggressiveness of symbiotic ants.

The number of attending ants per larva differed markedly between myrmecophyte-feeding *A. amphimuta* (6.8±0.97) and *A. dajagaka* (17.5±2.72). This is most likely to be due to the amount of honeydew secreted by the larvae, which is proportional to body size. Indeed, more honeydew is secreted from an *A. dajagaka* larva (droplets >1 mm in diameter) than from an *A. amphimuta* larva (droplets <0.5 mm in diameter). This is supported by a previous study that demonstrated that the amount of honeydew provided by the DNO of *Polyommatus coridon* (Lycaenidae) influenced the number of attending ants (Fiedler & Maschwitz, 1988, 1989).

Evolution of myrmecophily in *Arhopala* species feeding on *Macaranga*

Given the *Arhopala* phylogeny reported by Megens *et al.* (2005), it would be reasonable to assume that the common ancestor of the *A. amphimuta* subgroup, to which all five *Arhopala* species belong, invaded a plant-ant relationship or pre-relationship, using primitive myrmecophilous functions (DNO, TOs, and PCOs) mainly through honeydew secretion from the DNO. Thus, *A. amphimuta* and *A. dajagaka* (and likely *A. moolaiana*) would have had obligate myrmecophily and evolved without functional changes of the myrmecophilous organs. In addition, the ancestor of *A. zylda* might have specialized in myrmecophytic host plants and their symbiotic ants, but evolved lacking the DNO through non nectar-secretion tactics such as chemical camouflage or mimicry, thus resulting in the extant species.

In contrast, through its evolution, *A. major* may have reversed from an obligate to a facultative relationship, as suggested by Megens *et al.* (2005), with a wide range of non-myrmecophytic host plants and ant species that are not specific to myrmecophytes. The reason why the larvae of *A. major* retain the three basic myrmecophilous organs is that they need to tame ants that may potentially attack the larvae, since their host plants often produce

extrafloral nectaries on the leaf surface to attract a group of ant species that does not specialize in the plants. This is likely particularly true for the *A. amphimuta* subgroup.

Relationships between *Arhopala* species and their parasitoids

We observed that the four Bornean *Arhopala* species were parasitized by three insect species. As far as we know, this is the first time that these parasitoids have been recorded for these *Arhopala* butterflies. Although a dipteran fly, *Aplomya distincta* emerged from all *Arhopala* species, the parasitoid composition was slightly different between the three myrmecophyte-feeding and one non-myrmecophyte-feeding *Arhopala* species. Specifically, an ichneumonid wasp, *Xanthopimpla pumilio*, emerged from the former species, whereas a braconid wasp, *Apanteles* sp., only emerged from the latter. This host-preference of the two parasitoids may be associated with differences in ant aggressiveness between myrmecophytes and non-myrmecophytes. Ants living with myrmecophytic *Macaranga* are generally more aggressive than those inhabiting non-myrmecophytes. Indeed, our data show that *A. major* feeding on the non-myrmecophytes exhibited a higher percentage of parasitism (39%) than the three *Arhopala* species found on myrmecophytes (29 to 34%). However, the current data are insufficient to support this hypothesis, and additional fieldwork is necessary.

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摘　　要

好蟻性植物オオバギ属を食餌植物とするムラサキツバメ属の幼生期と生態 (大久保忠浩・矢後勝也・市岡孝朗)

トウダイグサ科オオバギ属 (*Macaranga*) のアリ植物種は, 植食者に対して防衛効果をもつ種特異的なアリ種に巣場所と食物を提供することによって, そのアリと共生し, 相利的な相互関係を結んでいる. そうした共生アリの対植食者防衛にもかかわらず, シジミチョウ科ムラサキツバメ属 (*Arhopala*) の一部を構成する *amphimuta* グループに属する種の幼虫がアリ植物オオバギを寄主植物としていることが知られている. オオバギ属を利用するムラサキツバメ属数種のマレー半島における寄主植物選択がこれまでに明らかにされているが, 幼生期における生活史の詳細は明らかにされていない.

そこで本研究では, ボルネオ島の低地フタバガキ原生林において, オオバギ属を利用するムラサキツバメ属4種の幼生期の形態, 行動, 寄主植物選択, 共生アリ, 捕食寄生者を記載した.

本調査地においては, 3種のムラサキツバメがそれぞれ1種または互いに近縁な2種のアリ植物オオバギ属のアリ植物種を寄主植物として利用していることが確認され, *Arhopala amphimuta* は *Macaranga trachyphylla* と *M. bancana* を, *A. zylda* は *M. beccariana* と *M. hypoleuca* を, *A. dajagaka* は *M. hosei* を, それぞれ寄主植物としていた. ボルネオにおける寄主植物選択を, Maschwitz *et al.* (1984) によって明らかにされているマレー半島のものと比較したところ, ボルネオにおけるオオバギ属とムラサキツバメ属の対応関係に見られる種特異性は高く, マレー半島のものと類似していた. また, 今回明らかにされた各アリ植物オオバギ属の種は特定のアリ種と共生関係を結んでいることが知られており, 本研究の観察でも同様な結果が得られたことから, 各ムラサキツバメ種に随伴する共生アリの種特異性もおそらく高いと考えられた. 一方, 別のムラサキツバメ属の1種 *A. major* は, *M. gigantea* を含む互いに近縁でない2種の非アリ共生型オオバギ属を食餌植物としており, 随伴するアリも不特定であったことから, 本種の寄主特異性は低いものと考えられた.

ムラサキツバメ属4種の幼虫や蛹の形態および色彩は, 各種が摂食あるいは静止する食餌植物の部位と見事に一致させており, 隠蔽色として十分働いているものと思われた. また, 捕食者から隠蔽するための幼虫の静止位置は, アリ植物種と非アリ植物種を寄主とするムラサキツバメ属の間に明確な違いが見られた.

ムラサキツバメ属4種の好蟻性器官は, 種により発達程度に違いが見られた. アリ植物種を寄主とする3種のうち, *A. amphimuta* と *A. dajagaka* は基礎的な好蟻性器官の DNO (蜜腺), TOs (伸縮突起), PCOs (pore cupola organs) をすべて備え, 各アリ植物の共生アリとの結びつきが強いが, *A. dajagaka* の方が体長は大きいために DNO も大きく, 多数のアリを随伴していた. *A. zylda* はアリ植物種を寄主とするにも関わらず, DNO が消失しており, アリの随伴性はかなり低い. ただし, 他の好蟻性器官は機能しているためか, アリ植物の共生アリに攻撃されない事実は注目に値する. 非アリ植物種を食す A major は3つすべての好蟻性器官を備えるが, DNO はあまり大きくないために分泌物の放出は少なく, 不特定のアリがいくらか集う程度であった.

Megens *et al.* (2005) によるムラサキツバメ亜族の系統樹から判断すると, *amphimuta* グループの共通祖先はおそらく好蟻性であり, 3種類の好蟻性器官を保持していたと考えられる. この祖先の一部が特殊なアリ植物種とその共生アリに適応あるいは共進化することにより, 合わせて好蟻性器官も変化しながら, 各現生種に分化してきたものと考えられた.

調査対象のムラサキツバメ属は3種の捕食寄生者によって寄生されていた. 寄生蠅の1種 *Aplomya dis-*

tincta は対象としたムラサキツバメ全4種を利用していたが, コマユバチの1種は *A. major* のみを利用しており, ヒメバチの1種 *Xanthopimpla pumilio* はアリ植物を寄主とする3種のムラサキツバメのみに寄生することが明らかにされた.

このように, 今回の調査対象とした4種のオオバギ食ムラサキツバメ属では, 好蟻性器官を含む幼虫の形態, 行動, 寄生捕食者の構成が種間で著しく異なっていた. それらの種間変異は寄主となっているオオバギ上のアリが示す攻撃性の違いと強く関連しているのではないかと推測された.

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